

IN THE CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A method of forming gradient loaded liposomes ~~having a lower inside/higher outside pH gradient~~, the method comprising:

(a) ~~contacting a solution of liposomes with a pharmaceutical agent~~ in an aqueous solution of up to about 60 mM of an acid with an anthracycline chemotherapeutic agent, at a temperature wherein the protonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the anthracycline chemotherapeutic agent by raising the pH of the solution to 5.0 or above;

(bc) cooling the solution to a temperature at which the unprotonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is not capable of permeating the membrane of the liposomes; and

(ed) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes ~~having a lower inside/higher outside pH gradient~~.

2. (Original) The method of claim 1 wherein the liposomes comprise phosphatidylcholine.

3. (Currently Amended) The method of claim 1 wherein the liposomes comprise phosphatidylcholine selected from the group of distearoylphosphatidylcholine, hydrogenated soy phosphatidylcholine, hydrogenated egg phosphatidylcholine, dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine, and dielaidoyl phosphatidyl choline.

4. (Original) The method of claim 1 wherein the liposomes further comprise cholesterol.

5. (Original) The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol.

6. (Original) The method of claim 1 wherein the liposomes further comprise non-phosphatidyl lipids.
7. (Original) The method of claim 6 wherein the non-phosphatidyl lipids comprise sphingomyelin.
8. (Original) The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol selected from the group of dimyristoylphosphatidylglycerol, dilaurylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, and distearoylphosphatidylglycerol.
9. (Original) The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol.
10. (Original) The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1:0.01 to about 1:1.
11. (Original) The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1.5:1.0 to about 3.0:1.0.
12. (Original) The method of claim 1 wherein the liposomes are unilamellar and less than about 100nm.
13. (Currently Amended) The method of claim 1 wherein the weight ratio of the liposomes to the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is up to about 200:1.
14. (Currently Amended) The method of claim 1 wherein the weight ratio of the liposomes to the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is about 1:1 to about 100:1.

15. (Currently Amended) The method of claim 1 wherein the weight ratio of the liposomes to the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is about 1:1 to about 50:1.

16. (Original) The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-2} .

17. (Original) The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-4} .

18. (Original) The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-5} .

19. (Original) The method of claim 1 wherein the acid has a permeability coefficient larger than about 1×10^{-4} cm/sec for the liposomes.

20. (Original) The method of claim 1 wherein the acid is selected from the group of formic acid, acetic acid, propanoic acid, butanoic acid, pentanoic acid, citric acid, oxalic acid, succinic acid, lactic acid, malic acid, tartaric acid, fumaric acid, benzoic acid, aconitic acid, veratric acid, phosphoric acid, sulfuric acid, and combinations thereof.

21. (Original) The method of claim 1 wherein the acid is citric acid.

22. (Original) The method in claim 1 wherein up to about 50 mM of an acid is employed.

23. (Currently Amended) The method of claim 1 wherein the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ exists in a charged state when dissolved in an aqueous medium.

24-29. (Canceled)

30. (Currently Amended) The method of claim 129 wherein the anthracycline chemotherapeutic agent is selected from the group of doxorubicin, epirubicin, and daunorubicin.

31-39. (Canceled)

40. (Original) The method of claim 1 wherein the temperature in step (a) is about 40°C to about 70°C.

41. (Original) The method of claim 1 wherein the temperature in step (a) is about 50°C to about 60°C.

42. (Currently Amended) The method of claim 1 wherein the solution is cooled in step (b) to a temperature of about 0°C to about 30°C.

43-46. (Canceled)

47. (Original) The method of claim 1 wherein the weak base is an ammonium salt having a mono- or multi-valent counterion.

48. (Original) The method of claim 1 wherein the weak base is selected from the group of ammonium sulfate, ammonium hydroxide, ammonium acetate, ammonium chloride, ammonium phosphate, ammonium citrate, ammonium succinate, ammonium lactobionate, ammonium carbonate, ammonium tartarate, ammonium oxalate, and combinations thereof.

49. (Original) The method of claim 1 wherein the weak base is alkyl-amine selected from the group of methyl amine, ethyl amine, diethyl amine, ethylene diamine, and propyl amine.

50. (Currently Amended) The method of claim 1 further comprising, during or after step (e), removing any unloaded pharmaceutical agent.

51. (Original) The method of claim 50 wherein the removing of the unloaded drug employs removing the unloaded drug via cross filtration or dialysis.
52. (Currently Amended) The method of claim 1 further comprising, after step (ed), dehydrating the liposomes.
53. (Original) The method of claim 52 wherein the dehydrating is carried out at a pressure of below about 1 atm.
54. (Original) The method of claim 52 wherein the dehydrating is carried out with prior freezing of the liposomes.
55. (Original) The method of claim 52 wherein the dehydrating is carried out in the presence of one or more protective monosaccharide sugars, one or more protective disaccharide sugars, or a combination thereof.
56. (Original) The method of claim 55 wherein the protective sugar is selected from the group of trehalose, sucrose, maltose, and lactose.
57. (Original) The method of claim 52 further comprising rehydrating the liposomes after the dehydrating.
58. (Original) The method of claim 1 wherein the liposomes are unilamellar vesicles.
59. (Original) The method of claim 1 wherein the liposomes are multilamellar vesicles.
60. (Original) The method of claim 1 wherein more than about 90 wt.% of the pharmaceutical agent is trapped in the liposomes.
61. (Currently Amended) The method of claim 1 further comprising, after step (ed), contacting the liposomes with a pharmaceutically acceptable carrier.

62. (Original) The method of claim 1 wherein the acid is present in about 20 mM to about 60 mM.

63. (Currently Amended) A method for preparing a pharmaceutical composition comprising:

(a) ~~contacting a solution of liposomes with a pharmaceutical agent~~ in an aqueous solution of up to about 60 mM of an acid with an anthracycline chemotherapeutic agent, at a temperature wherein the protonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the anthracycline chemotherapeutic agent by raising the pH of the solution to 5.0 or above;

(bc) cooling the solution to a temperature at which the unprotonated form of the anthracycline chemotherapeutic agent ~~pharmaceutical agent~~ is not capable of permeating the membrane of the liposomes;

(ed) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes; and

(de) combining the liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

64. (Original) A method comprising administering the pharmaceutical composition of claim 63 to a mammal.

65. (Currently Amended) A method for treating a mammal inflicted with cancer, the method comprising administering the pharmaceutical composition of claim 63 to the mammal, ~~wherein the pharmaceutical agent is an antineoplastic agent.~~

66. (Original) The method of claim 65 wherein the cancer is a tumor, ovarian cancer, small cell lung cancer (SCLC), non small cell lung cancer (NSCLC), leukemia, sarcoma, colorectal cancer, head cancer, neck cancer, or breast cancer.

67. (Currently Amended) The method of claim 65 wherein the administration of the anthracycline chemotherapeutic agent antineoplastic agent, via the liposomal formulation, has a toxicity profile that is lower than the toxicity profile associated with the administration of the anthracycline chemotherapeutic agent antineoplastic agent in the free form.

68. (Original) The method of claim 67 wherein the toxicity is selected from the group of gastrointestinal toxicity and cumulative dose-dependent irreversible cardiomyopathy.

69. (Currently Amended) The method of claim 65 wherein the administration of the anthracycline chemotherapeutic agent antineoplastic agent has unpleasant side-effects that are lower in incidence, severity, or a combination thereof, than unpleasant side-effects associated with the administration of the antineoplastic agent in the free form.

70. (Original) The method of claim 69 wherein the unpleasant side-effects are selected from the group of myelosuppression, alopecia, mucositis, nausea, vomiting, and anorexia.

71. (Currently Amended) A gradient loaded liposome ~~having a lower inside/higher outside pH gradient~~ prepared by the process comprising:

(a) ~~contacting a solution of liposomes with a pharmaceutical agent~~ in an aqueous solution of up to about 60 mM of an acid with an anthracycline chemotherapeutic agent, at a temperature wherein the protonated form of the anthracycline chemotherapeutic agent pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the anthracycline chemotherapeutic agent pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the anthracycline chemotherapeutic agent by raising the pH of the solution to 5.0 or above;

(bc) cooling the solution to a temperature at which the unprotonated form of the anthracycline chemotherapeutic agent pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(ed) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.